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Abnormalities in the NC1 domain of collagen type IV in GBM in canine hereditary nephritis

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Abnormalities in the NC1 domain of collagen type IV in GBM in canine hereditary nephritis. Samoyed hereditary glomerulopathy (SHG) in dogs serves as a model for human X-linked hereditary nephritis (HN). We previously showed that glomerular capillaries of affected males did not stain by immunofluorescence (IF) using serum from a patient with Goodpasture's syndrome. Our goal in the present study was to determine whether the NC1 domain of the collagen type IV molecule, which contains Goodpasture antigen (GPA), could be demonstrated in these dogs, and to assess its immunological reactivity. By SDS-PAGE, NC1 in collagenase digests of glomerular basement membranes (GBM) of unaffected and carrier female dogs in the family with SHG showed 24 kilodalton (kD), 26 kD and 28 kD monomer, and 46 kD and 47 kD dimer components, but the 24 kD monomer was diminished in the affected males. By IF, a rabbit antibody to NC1 stained glomerular capillaries of unaffected, affected male, and carrier female dogs. In contrast, a human anti-GBM plasmapheresis fluid (PPF) stained glomerular capillaries of only the unaffected and carrier female dogs. By RIA, both antibodies reacted strongly with NC1 in collagenase digests of GBM of the unaffected and carrier female dogs, but showed reduced reactivity with NC1 of affected males. By Western blotting, both antibodies bound to dimers and 24 kD and 26 kD monomers of the NC1 domain in collagenase digests of GBM of unaffected and carrier female dogs. However, in affected males, the rabbit anti-NC1 antibody did not bind to the 24 kD monomer, while the human anti-GBM PPF showed weak binding to the 24 kD and 26 kD monomers. Hence, although the NC1 domain could be detected in GBM of affected male dogs, a reduced amount of the 24 kD monomer was present and, as well, the 26 kD monomer possessed altered immunological reactivity. These two monomers are known to be derived from separate autosomal gene products in man. Hence, our studies raise the possibility that, in SHG and X-linked HN, the underlying defect may involve a protein which is coded on the X chromosome and is involved in modifying the collagen type IV molecule.

Hereditary nephritis (HN) is an inherited glomerular disease, often associated with extrarenal manifestations including deafness (Alport's syndrome) and anterior lenticonus [1, 2]. Most patients present with hematuria and later develop proteinuria. Males usually progress to end-stage renal disease, whereas females show a variable outcome, ranging from unimpaired renal function to renal failure. Widespread multilaminar splitting of glomerular capillary basement membranes is seen by electron microscopy (EM) [1–4].

Recent insight into the pathogenesis of HN has arisen from studies utilizing immunofluorescence (IF) microscopy performed on renal biopsies of patients with HN. When these were examined using serum obtained from patients with Goodpasture's syndrome, glomerular capillaries of most males and some females with HN failed to stain, while staining was seen in healthy subjects and patients with glomerular diseases other than HN [5–8]. It has been proposed that a constituent of glomerular capillaries, referred to as Goodpasture antigen (GPA), is lacking in HN patients and that it plays a role in the development of HN. However, further studies on pathogenesis have been hindered by the absence of a suitable animal model for HN.

We previously described the clinical, genetic, and morphologic features of a spontaneously occurring renal disease in a family of Samoyed dogs, termed Samoyed hereditary glomerulopathy (SHG), which closely resembles HN in man [9–12]. In these studies, the gene for SHG was shown to be inherited in an X-linked dominant fashion [9], so that males with SHG develop severe renal disease and females show only mild renal impairment [12]. By EM, glomerular capillary basement membranes of affected males demonstrate extensive multilaminar splitting, identical to that in human HN, while carrier females show only segmental areas of splitting [10]. Moreover, glomerular capillaries of affected male dogs did not stain by IF using serum from a patient with Goodpasture's syndrome that cross reacted with normal dog kidney, implying that GPA was absent or immunologically altered [11]. In the present study, the NC1 domain of the collagen type IV molecule, which contains GPA, could be demonstrated by SDS-PAGE in collagenase digests of glomerular basement membranes (GBM), not only of unaffected and carrier female dogs in the pedigree with SHG, but also of affected males. However, the 24 kilodalton (kD) component of NC1 in GBM of affected male dogs was diminished in amount, as assessed by SDS-PAGE. Furthermore, the 26 kD component showed altered immunological reactivity, as assessed by Western blotting. These findings have allowed us to speculate on the pathogenesis of human HN.

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Methods

Classification of Samoyed dogs

Offspring of carrier females in a family with SHG were sacrificed at five months of age. Kidneys were removed and stored at −20°C until used for extraction of NC1 from GBM. Portions of kidneys of the Samoyed dogs were also fixed in 4% paraformaldehyde-1% glutaraldehyde and processed for EM. The Samoyed dogs were classified as unaffected, affected males, or carrier females on the basis of the EM appearance of their glomerular capillary basement membranes (that is, normal in unaffected dogs, extensive multilaminar splitting in affected males and segmental multilaminar splitting in carrier females) [10]. Based on this study, the kidneys were pooled into three groups.

Preparation of collagenase digests of GBM

Glomeruli were obtained by graded sieving, as previously described [13], from the kidneys of adult mixed breed dogs at the time of sacrifice following experimental cardiovascular surgery, from the Samoyed dogs, and from humans 3 to 17 years of age at the time of post-mortem examination. Briefly, portions of renal cortex were forced through a 115 mesh stainless steel sieve (WS Tyler, St. Catharines, Ontario, Canada) and suspended in normal saline. The suspension was then passed through an 80 mesh sieve seated above a 150 mesh sieve. Glomeruli were collected from the 150 mesh sieve, washed in normal saline followed by distilled water and centrifuged into a pellet. The pellet was suspended in 1 M NaCl and sonicated until it no longer contained cellular components but consisted only of GBM, as assessed by light microscopy. The sonicate was washed five times with 1 M NaCl, five times with distilled water, centrifuged into a pellet, and lyophilized. The sonicate was then weighed and suspended at a concentration of 5 mg/ml in 0.05 M HEPES buffer, pH 7.5, containing 1 mM phenylmethanesulphonylfluoride and 0.01 M $CaCl_2$. Bacterial collagenase type VII (Sigma Chemical Co., St. Louis, Missouri, USA) was added (10 U/mg GBM) and incubation was carried out for 72 hours at 37°C with constant agitation. The preparation was then centrifuged for one hour at 100,000 g and the supernatant was precipitated overnight by adding a tenfold volume of 95% ethanol. The precipitate was centrifuged into a pellet, air dried, and stored at −20°C.

Rabbit and human antibodies

Rabbit anti-NC1 antiserum. A collagenase digest was prepared from GBM of mixed breed dogs and was subjected to SDS-PAGE. The gel was stained with Coomassie Brilliant Blue, the NC1 dimer and monomer bands were cut out, pooled, destained with ethanol, crushed, and emulsified in complete Freund's adjuvant. A rabbit was injected subcutaneously at monthly intervals with 1 mg of this preparation and bled after the third injection. This reagent has been referred to as 'rabbit anti-NC1 antiserum' in the present study.

Human anti-GBM plasmapheresis fluid (PPF). PPF containing antibody to human GBM that cross reacted with dog glomerular capillaries, as assessed by IF, was obtained from a patient with anti-GBM nephritis who was being treated by plasmapheresis. It has been referred to in this study as 'human anti-GBM PPF'.

IF microscopy

Tissue sections of kidney were snap frozen in liquid nitrogen, stored at −70°C, and cut at 5 μ on a cryostat. The sections were picked up on gelatinized slides, treated with rabbit anti-NC1 antiserum (1:20 dilution) or human anti-GBM PPF (1:3 dilution) and stained using either fluoresceinated goat anti-rabbit (Miles, Naperville, Illinois, USA) or rabbit anti-human (Calbiochem, San Diego, California, USA) IgG antisera. In some experiments, before the rabbit antiserum or human PPF and fluoresceinated conjugates were applied, the tissue sections of kidney were first treated for two hours with acid urea (AU) (6 M urea, 0.1 M glycine-HCl, pH 3.5) to expose hidden NC1, as previously described [14]. Normal rabbit serum and PPF obtained from a patient being treated for familial hypercholesterolemia were used as negative controls.

SDS-PAGE

SDS-PAGE was performed according to the method of Laemmli [15], using SDS-containing 5% stacking and 8 to 18% gradient running polyacrylamide slab gels. Fifty μg of collagenase digest were applied to each lane and the gels were stained with Coomassie Brilliant Blue. Standards (Sigma) used to calculate molecular weights included phosphorylase B (92.5 kD), bovine serum albumin (69 kD), ovalbumin (46 kD), carbonic anhydrase (30 kD), soybean trypsin inhibitor (21.5 kD) and lysozyme (14.3 kD).

Western blotting

Western blotting was performed according to the method of Towbin, Staehelin and Gordon [16]. Gels were subjected to SDS-PAGE, using 25 μg of collagenase digest in each lane, and the separated components were transferred to nitrocellulose paper at 25 volts for 18 hours using a Trans-blot cell (Biorad, Richmond, California, USA). The blots were then incubated for six hours in 3% bovine serum albumin in Tris buffered saline, pH 7.4, and overnight in a 1:100 dilution of rabbit anti-NC1 antiserum or a 1:50 dilution of human anti-GBM PPF. The blots were rinsed and incubated for five hours with 10^6 cpm I^{125} protein A, exposed to X-ray film and developed.

Plate-binding RIA

The rabbit anti-NC1 antiserum and human anti-GBM PPF were tested in a plate-binding RIA for reactivity against dog NC1, laminin, and collagen type IV. Negative controls were the normal rabbit serum and PPF used in the IF experiments. Rabbit anti-laminin antiserum was obtained from Bethesda Research Lab (Gaithersburg, Maryland, USA), while rabbit anti-collagen type IV antiserum was prepared by us by immunizing rabbits with triple-helical collagen type IV extracted from human placenta by pepsin digestion [17]. Laminin was obtained from Bethesda Research Lab, collagen type IV was prepared from a pepsin digest of placenta and obtained from Calbiochem (San Diego, California, USA), and NC1 was prepared by us by collagenase digestion of GBM. Wells of disposable flexible microtiter plates (Limbro 76-364-05, Flow Laboratories, McLean, Virginia, USA) were coated for two hours at 37°C with 100 μl of the various substrates (10 μg/ml) plated in 0.02 M phosphate-buffered saline (PBS) (145 mM NaCl, 9 mM NaH_2PO_4, 11 mM Na_2HPO_4, pH 7.2) or AU. The substrates

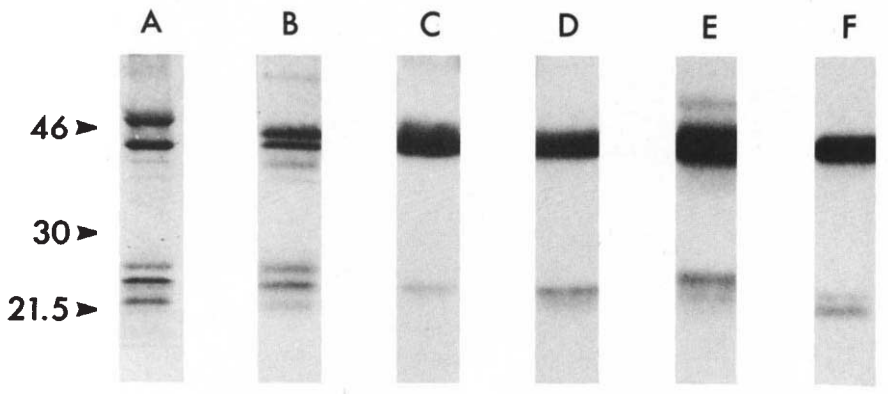

Fig. 1. *SDS-PAGE and Western blotting of NC1 in collagenase digests of GBM of humans and mixed breed dogs.* In a first experiment, 50 μg samples of collagenase digest of GBM of **(A)** humans and **(B)** mixed breed dogs were examined by SDS-PAGE, as outlined in **Methods**. The gel was stained with Coomassie Brilliant Blue. Molecular weights of standards are shown at the left. In a second experiment, 25 μg samples of human **(C and E)** and dog **(D and F)** collagenase digest were subjected to SDS-PAGE, then transferred to nitrocellulose (Western blotting) and developed using rabbit anti-NC1 antiserum (C and D) or human anti-GBM-PPF (E and F), as outlined in **Methods.**

were then removed. The wells were washed three times with PBS and flooded with 10% fetal calf serum for two hours at 37°C, after which the fetal calf serum was removed and the wells were again rinsed three times with PBS. Then, 100 μl dilutions of the rabbit anti-NC1 antiserum or human anti-GBM PPF was added to the wells and the plates were incubated for 16 hours at 4°C, at which time these reagents were removed and the wells were washed three times with PBS. Finally, 100 μl of I^{125} protein A (Amersham, Arlington Heights, Illinois, USA), containing 20,000 cpm, was added to the wells for two hours at room temperature. The wells were washed three times with PBS, cut out and counted on a Beckman 300 gamma counter.

Results

Analysis by SDS-PAGE of NC1 domain in collagenase digests of GBM of humans, mixed breed dogs and Samoyed dogs

By SDS-PAGE, the NC1 domain in collagenase digests of GBM of humans showed monomer bands at 24 kD, 26 kD and 28 kD, and dimer bands at 46 kD and 48 kD (Fig. 1A). The pattern of NC1 obtained from GBM of mixed breed dogs was similar except that the dimer bands were at 46 kD and 47 kD (Fig. 1B). By SDS-PAGE, NC1 in collagenase digests of GBM of unaffected and carrier female dogs of the pedigree with SHG showed similar bands to those seen in the mixed breed dogs (Fig. 2A,C). However, in the affected males, there was a diminution in the amount of the 24 kD monomer band (Fig. 2B).

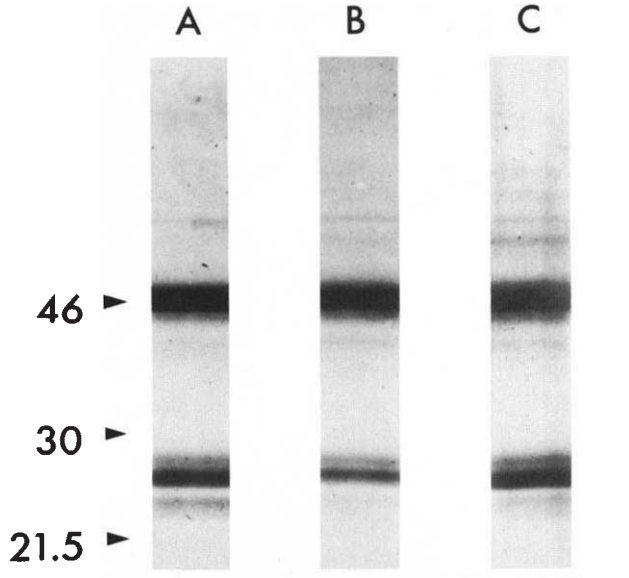

Fig. 2. *SDS-PAGE of 50 μg samples of collagenase digests of GBM of unaffected* **(A)**, *affected male* **(B)**, *and carrier female* **(C)** *Samoyed dogs.* SDS-PAGE was performed as outlined in **Methods**. The gel was stained with Coomassie Brilliant Blue. Molecular weights of standards are shown at the left.

Characterization of the rabbit and human antibodies

Rabbit anti-NC1 antiserum. By IF, the rabbit anti-NC1 antiserum failed to stain glomerular capillaries on tissue sections of normal dog kidney before treatment with AU. However, following AU, strong staining was observed, indicating that this antiserum detected hidden NC1 determinants (Fig. 4). Using the RIA, this antiserum reacted only weakly with NC1 obtained from GBM of mixed breed dogs when the NC1 was plated in PBS, but reactivity was markedly enhanced when the NC1 was plated in AU (Fig. 3A). The rabbit anti-NC1 antiserum did not react by RIA with laminin (Fig. 3B) or collagen type IV (data not shown). In the case of laminin and collagen type IV, no enhancement of binding was observed when these substrates were plated in AU prior to reaction with their respective antisera (data not shown). By Western blotting, the rabbit anti-NC1 antiserum bound to the dimer and 26 kD monomer components of human NC1 (Fig. 1C) and to the dimer and 24 kD and 26 kD components of dog NC1 (Fig. 1D).

Human anti-GBM PPF. By IF, the human anti-GBM PPF also showed no staining of glomerular capillaries before, but strong staining after, treatment of tissue sections of normal dog kidney with AU, indicating that it detected hidden NC1 determinants (Fig. 4). By RIA, the PPF reacted with dog NC1, but only when the NC1 was plated in AU (Fig. 3C). No reactivity by RIA was seen with laminin or collagen type IV (data not shown). By Western blotting, binding to the dimer and all three monomer components of human NC1 was seen (Fig. 1E). The strongest binding was to the 28 kD monomer, with intermediate binding to the 26 kD monomer, and only weak binding to the 24 kD monomer. With dog NC1, binding to the dimers and 24 kD and 26 kD monomers was observed, with stronger binding to the 24 kD than to the 26 kD monomer (Fig. 1F).

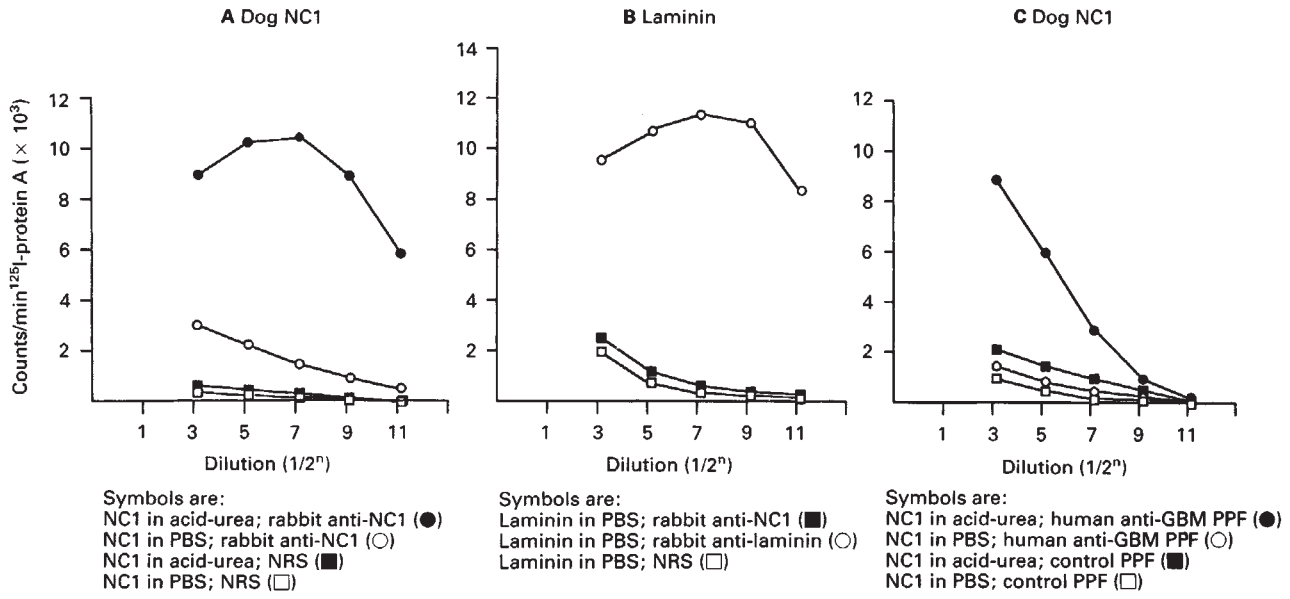

Fig. 3. *Plate binding radioimmunoassay, showing reactivity of rabbit antiserum to NC1 with* **(A)** *dog NC1 and* **(B)** *laminin, and reactivity of human anti-GBM PPF with* **(C)** *dog NC1.* Wells of microtiter plates were coated with a collagenase digest obtained from dog GBM (dog NC1) or laminin, followed by rabbit antiserum (A and B) or human PPF (C), and I^{125} protein A, as outlined in **Methods**. Collagenase digest was plated either in acid urea or PBS, while laminin was plated only in PBS.

Characterization of immunological reactivity of NC1 domain in tissue sections of kidney and collagenase digests of GBM of Samoyed dogs

IF microscopy. With the rabbit anti-NC1 antiserum, no staining of glomerular capillaries of any of the Samoyed dogs was seen before treatment of tissue sections of kidney with AU (Fig. 4A). Following AU, staining was seen in unaffected (Fig. 4B), affected male (Fig. 4C) and carrier female dogs. In the case of the human anti-GBM PPF, there was also no staining of glomerular capillaries of unaffected (Fig. 4D), affected male, or carrier female dogs before treatment with AU. However, following AU, glomerular capillaries of unaffected (Fig. 4E) and carrier female dogs became positive, but those of affected males remained negative (Fig. 4F).

Plate-binding RIA. The rabbit anti-NC1 antiserum reacted strongly with NC1 in collagenase digests of GBM of unaffected and carrier female Samoyed dogs, especially when the NC1 was plated in AU (Fig. 5A–C). Reactivity was somewhat diminished with NC1 in GBM of affected males. In the case of the human anti-GBM PPF, reactivity with NC1 was seen only after treatment with AU. Strong reactivity was detected with the NC1 of unaffected and carrier female dogs, but only weak reactivity was seen with NC1 of affected male dogs (Fig. 5D–F).

Western blotting. By Western blotting, the rabbit anti-NC1 antiserum bound to the dimer and 24 kD and 26 kD monomer components of NC1 in collagenase digests of GBM of unaffected (Fig. 6A) and carrier female (Fig. 6C) Samoyed dogs. Similar results were seen with the affected males, except that no binding to the 24 kD component of NC1 was observed (Fig. 6B). The human anti-GBM PPF bound strongly to the dimer and 24 kD and 26 kD monomer components of NC1 in collagenase digests of GBM of unaffected (Fig. 6D) and carrier female (Fig. 6F) dogs, but only weakly to the 24 kD and 26 kD monomer components of affected male dogs (Fig. 6E).

Discussion

Collagen type IV is composed of four domains: a main triple helix, a separate triple helix at the N-terminal end, referred to as the 7S domain, and two non-collagenous (NC) regions. Of these, the NC2 region joins the two triple helical portions, while the NC1 region is located at the C-terminal end of the molecule [18–23]. The NC1 domain obtained by collagenase digestion has been isolated and found to contain GPA, as demonstrated by its reactivity with serum obtained from patients with Goodpasture's syndrome [18, 22–24]. NC1 has a molecular weight of about 170K [22, 25] and is a hexamer, with contributions from both the α1(IV) and α2(IV) chains of collagen type IV [22, 25–27] and possibly from additional chains [27]. Following denaturation, NC1 dissociates into dimers and monomers, which are homologous in amino acid composition, and yields the two sets of bands by SDS-PAGE [24].

We initially characterized NC1 in collagenase digests of GBM of mixed breed dogs before turning to the family of dogs with SHG. Previous studies in the literature have utilized mainly human and bovine material, and data are not available on dogs. SDS-PAGE of non-reduced collagenase digests of human and bovine GBM has revealed bands in the region of 48 to 54 kD and 22 to 30 kD [23–26]. However, the size and number of bands have varied in different studies, depending on the starting material and the conditions of electrophoresis. It has recently been reported that NC1 isolated from human GBM under non-reducing conditions could be resolved into three bands in the lower molecular weight region, located at 24 kD, 26 kD, and 28 kD [28, 29]. Dog NC1 was compared with human NC1 using

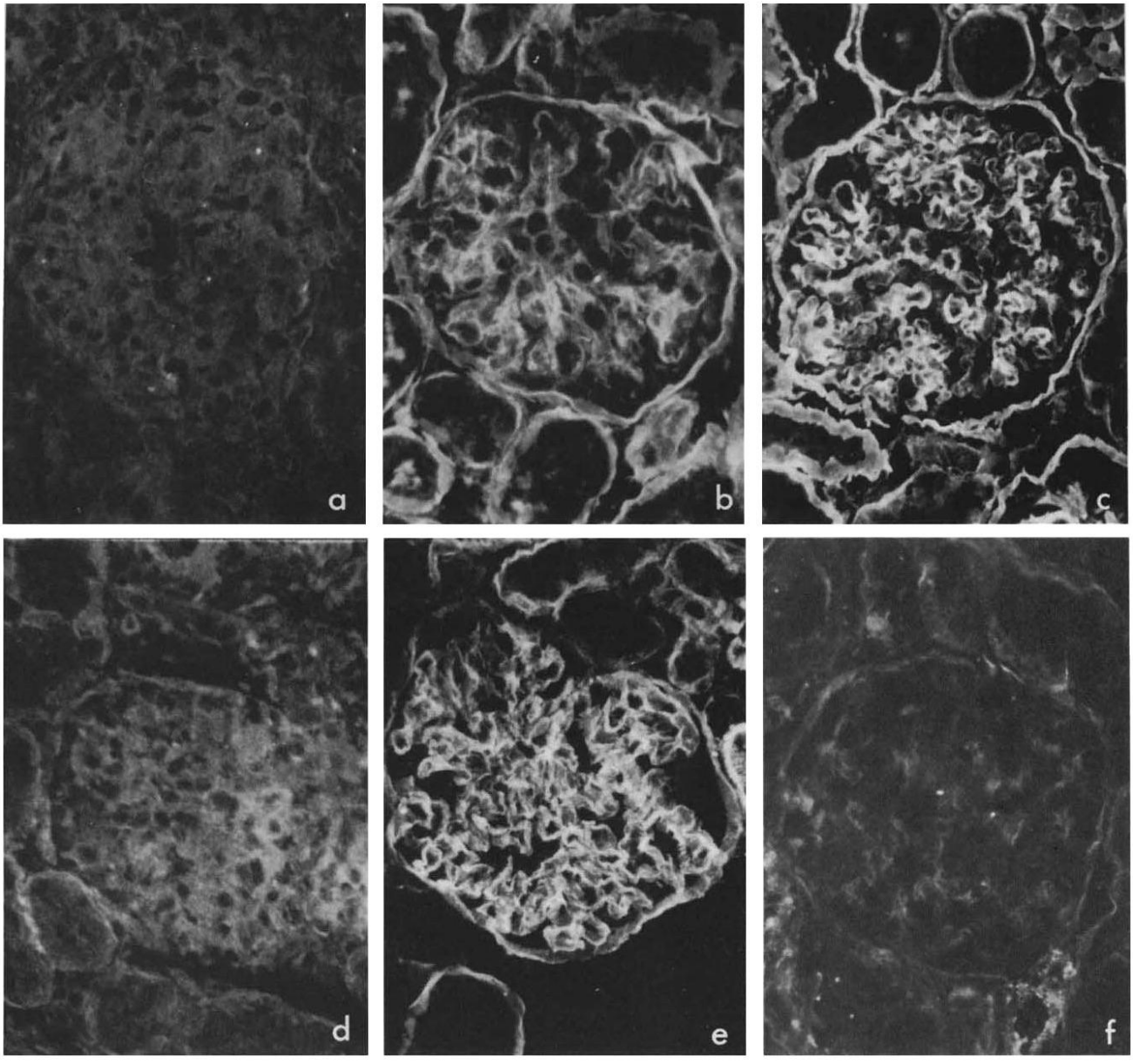

Fig. 4. *Immunofluorescence (IF) microscopy of glomeruli of unaffected* **(a,b,d,e)** *and affected male Samoyed dogs* **(c,f)** *using rabbit anti-NC1 antiserum* **(a,b,c)** *and human anti-GBM PPF* **(d,e,f)**. IF microscopy was performed, as described in **Methods**, using tissue sections which were either not pretreated with acid urea (a,d) or pretreated with acid urea to expose hidden NC1 determinants (b,c,e,f). (× 300).

these designated molecular weights. In this respect, dog NC1 obtained from GBM resembled human NC1 by SDS-PAGE, except for a 47 kD instead of a 48 kD dimer band.

Both of the antibodies used in this study were characterized by IF, using tissue sections of kidney of mixed breed dogs, and by RIA and Western blotting, using NC1 obtained by collagenase digestion of GBM of these dogs. Although both produced staining of glomerular capillaries by IF, staining was evident only after treatment of the tissue sections with AU, indicating that the antibodies detected hidden NC1 determinants [14]. Moreover, these antibodies reacted by RIA with dog NC1 obtained from GBM. Reactivity was enhanced by plating in AU, in the case of the rabbit anti-NC1 antiserum, and observed only in the presence of AU, in the case of the human anti-GBM PPF. Finally, by Western blotting, both reagents bound to the 24 kD and 26 kD components of dog NC1. In this respect, they differed in their binding to human NC1, in which the rabbit anti-NC1 antiserum bound only to the 26 kD monomer, and the human anti-GBM PPF bound to all three monomers, but mainly to the 28 kD monomer. Western blotting using serum from patients with Goodpasture's syndrome has been performed by many investigators on NC1 isolated from normal human GBM [23, 25, 30, 31]. GPA reactivity has been shown to reside within the 28 kD monomer of human NC1 [28, 29]. The inability of both of our anti-NC1 antibodies to bind to the 28 kD monomer of dog NC1 means that they are not useful for assessing the immunological reactivity of this monomer in the family of dogs with SHG. As well, these data imply that the 28 kD bands of

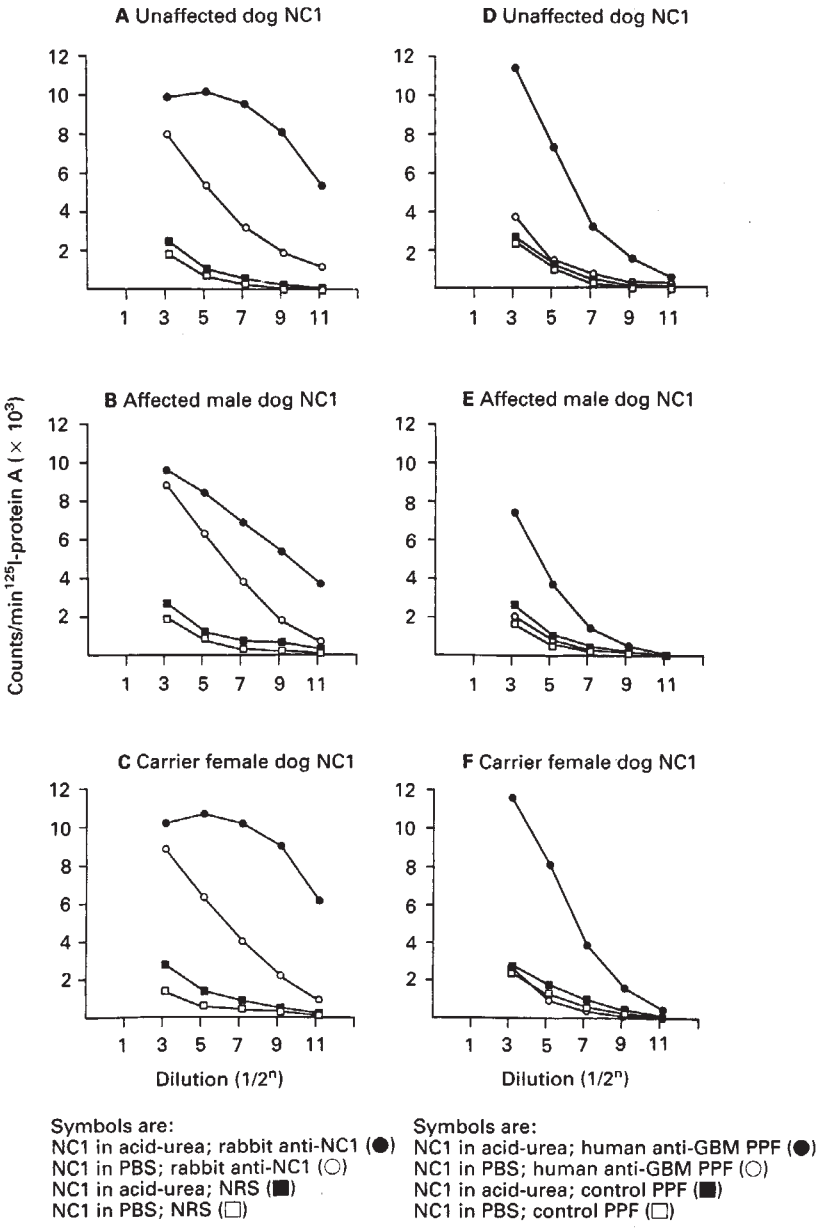

Fig. 5. *Plate binding radioimmunoassay, showing reactivity of rabbit anti-NC1 antiserum and human anti-GBM PPF with NC1 in collagenase digests of GBM of unaffected* **(A,D)**, *affected male* **(B,E)**, *and carrier female* **(C,F)** *Samoyed dogs.* Wells of microtiter plates were coated with the collagenase digests, plated in either acid urea or PBS, followed by rabbit anti-NC1 antiserum (A,B and C) or human anti-GBM PPF (D,E and F) and I^{125} protein A, as outlined in **Methods**.

human and dog are not equivalent. This view has been reinforced by the observation that the strongest binding by human anti-GBM PPF to human NC1 was to the 28 kD band, whereas in dog NC1, the strongest binding was to the 24 kD band. The 24 kD band in dog NC1 may be equivalent to the 28 kD band in human NC1, and thus may contain GPA.

Studies were next performed on NC1 in collagenase digests of GBM of dogs in the SHG pedigree. Similar 24 kD, 26 kD and 28 kD monomer and 46 kD and 47 kD dimer bands were detected by SDS-PAGE in collagenase digests of unaffected and carrier female dogs. NC1 could also be isolated from GBM of affected males, but it differed in that the 24 kD monomer was diminished in amount. Examination of NC1 in GBM of patients with the X-linked form of HN by SDS-PAGE has demonstrated a loss of the 28 kD band [32]. Hence, it would appear that different defects in the NC1 domain are present in SHG in dogs and HN in man. However, if the 28 kD human band is the equivalent of the 24 kD dog band, as mentioned above, then the defect in the NC1 domain may actually be similar in SHG and HN.

In addition to the abnormality demonstrated by SDS-PAGE, a further difference in the NC1 domain in collagenase digests of GBM of affected male dogs was demonstrated on the basis of immunological reactivity, using IF, RIA, and Western blotting. Glomerular capillaries of affected male dogs failed to stain by IF with the human anti-GBM PPF, and NC1 obtained from GBM of these dogs reacted by RIA only poorly with the human anti-GBM PPF. Moreover, the 24 kD monomer of NC1 in collagenase digests of GBM of affected male dogs failed to bind to the rabbit anti-NC1 antiserum by Western blotting, while the 24 kD and 26 kD monomers bound only weakly to the human anti-GBM PPF. Although the decreased binding by both these

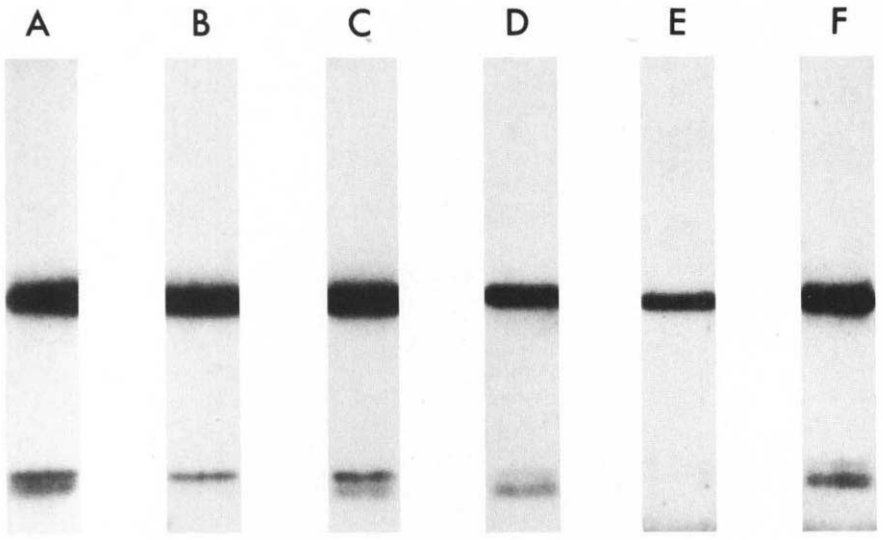

Fig. 6. *Western blotting of 25 μg samples of collagenase digests of GBM of unaffected* **(A,D)**, *affected male* **(B,E)**, *and carrier female* **(C,F)** *Samoyed dogs.* Collagenase digests were analyzed by SDS-PAGE, transferred to nitrocellulose (Western blotting) and developed using rabbit anti-NC1 antiserum (A,B,C) or human anti-GBM PPF (D,E,F), as outlined in **Methods**.

antibodies to the 24 kD component could be attributed to a reduced amount of this monomer, as demonstrated by SDS-PAGE, these observations suggest that the NC1 domain in collagenase digests of GBM of affected males was immunologically altered in its 26 kD monomer component. A defect in the 28 kD monomer of NC1 of affected male dogs could not be excluded, since neither of the two anti-NC1 reagents used in the present study reacted with 28 kD monomer. Using 2-D gel electrophoresis and Western blotting of normal human GBM, the greatest binding of serum from patients with Goodpasture's syndrome was to the cationic proteins of NC1 [28, 31], particularly to the cationic 28 kD subunit [28], with weaker binding to the 24 kD, 26 kD, and non-cationic 28 kD subunits of NC1. Both the cationic and non-cationic 28 kD components have been reported to be absent from GBM of patients with HN [32]. Some patients with HN who have undergone renal transplantation have developed anti-GBM antibodies, which, by IF, bound to glomerular capillaries [33]. However, serum from a patient with HN who developed anti-GBM nephritis following renal transplantation showed binding to the 26 kD but not to the 28 kD component of NC1 [34]. Nevertheless, another such serum showed binding to the 28 kD band [32]. All of the foregoing results suggest that the abnormality in HN could involve either the 26 kD or 28 kD monomer components of NC1. Our results of SDS-PAGE and Western blotting of NC1 in GBM of affected male dogs with SHG suggest that both the 24 kD and 26 kD components are abnormal.

Considerable work has been done to elucidate the molecular nature of the NC1 domain using human and bovine material. The dimer and monomer bands seen by SDS-PAGE are derived from both the α1(IV) and α2(IV) chains of collagen type IV [22, 25–27, 30, 35]. Specifically, the monomer bands seen at 26 kD and 24 kD are derived from the α1(IV) and α2(IV) chains, respectively. A third monomer, with a molecular weight of about 28 kD, has also been recovered; its source is unknown, but it may be a third chain of collagen type IV or a post-translational modification product of the α1(IV) or α2(IV) chains [25–29]. The genes for both the α1(IV) and α2(IV) chains have been localized to chromosome 13 in man [36, 37], rather than to the X chromosome, on which the SHG gene and the gene for X-linked HN are located [1, 2]. Since, at least in man, the 24 kD and 26 kD monomers are derived from separate autosomal gene products, it is difficult to reconcile the existence of abnormalities in both these monomers, as seen in SHG, with a single structural gene defect. Rather, our results suggest the possibility that there is a defect in a protein coded on the X chromosome which is involved in modifying both these monomers. A defect in an enzyme modifying collagen type IV has also been postulated as a possible mechanism underlying human HN [32]. Further work is being carried out to investigate this hypothesis, and to establish whether there is a role for a third type of collagen type IV chain in SHG. It is still possible that different defects underlie SHG and HN but produce the same result, namely, a disturbance of the function of the NC1 domain. The NC1 region is involved in the initiation of triple helix formation of the collagen type IV molecule [19] and in the cross linking of collagen type IV molecules in the supramolecular assembly of basement membranes [20, 22, 23, 38]. A defect in the NC1 domain, whatever its cause, could lead to faulty GBM, as seen both functionally and ultrastructurally, in dogs with SHG and patients with HN. However, the exact molecular basis for the defect remains to be clarified.

Acknowledgments

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